1. INTRODUCTION

Aquaculture has emerged as one of the most gifted and fastest growing sector and provides premium animal protein, raises nutritional levels and generates income and employment around the globe (FAO, 2010). The World fish production achieved during 2013-2014 is 8.03 million metric tones and the contribution of fish from inland sector alone is 5.07 million metric tones which is about 63 % of overall fish production (IFAI, 2013). On the other hand, ornamental fish culture is upcoming chief branch of aquaculture and gaining importance due to its incredible economic opportunities and prospects. Rearing or holding ornamental fish gives an immense pleasure and peace. The global ornamental fish trade is a multimillion dollar industry that sustains thousands of rural people in the growing nations. Ornamental fish culture is an emerging industrial sector, which requires continued research with scientific and technical developments and innovation.

1.1. Ornamental fish culture

Ornamental fishes are called “living jewels” as they are attractive, peaceful, mostly tiny and capable of living in small confined areas. Aquarium fishes are probably the most cheerful living creatures and emerged as the second most popular hobby next to photography (Chapman, 1997). With immense popularity of aquarium keeping in public places and households, ornamental fish culture has resulted the higher production throughout the World and their trading has become lucrative over the years. At present approximately 850 ornamental fish species come from marine resources and 750 species from freshwater (Olivier, 2001). Initially, ornamental fish keeping is largely practised in the developed countries. However, in recent times, it is also gaining importance in developing countries. It is well known that the Western
Ghats of India is one of the 34-Biodiversity ‘hotspot’ areas of the World (Anna Mercy, 2007). Approximately, 300 species of freshwater fishes have been recorded in the Western Ghats, out of which 155 species are considered as ornamental fishes, of which 117 are endemic to the Western Ghats. During the last few decades there has been considerable growth and diversification in the international trade.

1.2. Global and national trade for ornamental fish

Global ornamental fish trade is a multibillion dollar industry. Since 1985, the international trade value of ornamental fish has been augmented at an average growth rate of 14 % per year (FAO, 2013). The global annual revenue of ornamental fish trade is about US $ 10 billion (Prathvi et al., 2013). European nations and Japan are the largest markets for ornamental fish but more than 65 % of the exports to these countries come from Asia (Felix, 2009). Indonesia, Singapore, China, Malaysia and Japan are the leaders in breeding and farming of freshwater ornamental fishes. World trade of ornamental fish is estimated to be about ` 2000 crores, however, India shares only ` 15 crores and is dominated by freshwater ornamental fishes (Raja et al., 2014).

The major export market for the Indian ornamental fish traders are Singapore, United States, United Kingdom, Belgium, Italy, Japan, China, Australia and South Africa. India’s overall ornamental fish trade is about 1.06 million US $ during the year 2009 (http://comtrade.un.org/db/dqBasicQuery.aspx. 21 Mar, 2014).

In India, among the freshwater ornamental fish trade, 98 % are cultured and 2 % from capture fishery. About 90 % of Indian aquarium fishes are distributed from Kolkata, followed by 8 % from Mumbai and 2 % from Chennai (Felix, 2009). Bengaluru alone is having more than 1,200 outlets of ornamental fish aquarium and it
generates revenue of `1.5 crores per month. The vast majority of ornamental fishes in
the aquarium trade are of freshwater origin and farm raised. Only 2% of total
ornamental fish trade are from marine fishes of which 98% are capture fishery and 2%
from production. Indian Western Ghats and North Eastern hills have rich diversity
of freshwater fishes (Anna Mercy, 2007). Among the 300 species of freshwater fishes
in the Western Ghats, only 114 species are exported (Anon, 2005). On the other hand,
out of 274 species, 32% of native fishes are distributed from North Eastern hill states
(Mahapatra et al., 2007). However, majority of the ornamental fishes marketed from
India are exotic species (Arif et al., 2015). In addition, considerable numbers of
indigenous species of high value ornamental fishes are also contributed greatly for the
development of Indian ornamental fish industry (Ghosh et al., 2003).

1.3. Indian ornamental fish varieties

Among the 287 native Indian fish species, 239 are freshwater fish and 48 are
marine fish species. About 80% of ornamental fish are from freshwater and the rest
from brackish and marine waters. Exotic fish are dominated in the domestic market.
Among these, 19 species belong to the family Cyprinidae, 9 species of Cichlidae and
5 species of Osphronemidae family (Jayalal and Ramachandran, 2012). Even though
there are quite a lot of indigenous fishes, having high potential as ornamental fishes,
they have not been properly exploited for the World market. Among the indigenous
freshwater species, 98 species belong to the Cyprinidae and 16 species fall under
Bagridae and Balitoridae and 12 species belong to the families Channidae, Cobitidae
and Sisoridae.

Most commonly, ornamental fish comprise two broad categories: live-bearers
and egg-layers. The common exotic live bearers are Poecilia reticulata (Guppy),
Poecilia latipinna (Molly), Xiphophorus helleri (Swordtail), Xiphophorus maculates (Platy) and egg layers are Carrassius auratus (Gold fish), Cyprinus carpio (Koi), Puntius tetrazona (Tiger barb), Betta splendens (Siamese fighter), Hyphessobrycon serape (Serpae tetra) and other Cichlid fishes. In addition to these, few imported fish such as Balantiocheilus melanopterus (Silver shark), Pterophyllum scalare (Angel), Labeo bicolor (Red tailed black shark) Labeo erythrurus (Red finned shark), Cyprinus carpio (Koi carp) and Flower horn are the most popular freshwater ornamental fish distributed in India in recent days (Raja et al., 2014).

Koi carp (Cyprinus carpio L.) is the biggest group of cultured fish and probably the most widely spread species. Carp comes under the family Cyprinidae, the term “Koi” comes from the Japanese word, Nishikigoi, meaning coloured or brocaded carp. Koi originates from common carp through mutation and is considered to be the most popular and attractive fish among the garden pond keepers and hobbyists. Worldwide, Koi is an economically important ornamental fish because of its numerous colours and colour combination (Jha and Barat, 2005). Some of the major colours are red, black, white, blue, cream and yellow and combination of some of these colours, which gives immense pleasure to the viewers. Carp is a large group of fish originally found in Central Europe and Asia. Still Japanese breeders produced very attractive Koi in the World. Some of the Japanese Koi varieties measure as long as 70 cm and fetch prices as high as US $ 15,000.

Cichlidae is one of the biggest families with more than 2000 types and new species are being exported every year (http://animal-world.com/encyclo/fresh /cichlid/cichlids.html). They are very gorgeous with widest diversity of body shapes, vast range of sizes and some vivacious colour with patterning. All these traits make
Cichlids as a favourite aquarium fish. This fish is categorized as “secondary freshwater fish” because their ancestors are from the sea. Few species of Cichlids are found in Asia. About 19 species belonging to the family Cichlidae have been exported from India (Jayalal and Ramachandran, 2012). The most popular Cichlid verities distributed in India are *Astronotus ocellatus*, *Cichlasoma severum*, *Hemichromis paynei*, *Herichthus cyanoguttatus*, *Julidochromis* sp., *Pseudotropheus greshakei*, *Pseudotropheus zebra*, *Pterophyllum scalare*, *Thorichthys meeki*, *Cichlasoma meeki*, *Papiliochromis ramirezi*, *Cichlasoma nigrofasciatum* and *Melanochromis auratus* (Jayalal and Ramachandran, 2012; Raja et al., 2014).

1.4. **Constrains in Indian ornamental fish industry**

In recent days, the Indian ornamental fish trading is gradually increasing and this is the appropriate time to hold the position and competitiveness in the World market. In general, trade might be adversely affected through the introduction of non-native organisms and by the direct depletion of wild stocks (Andrews, 1990). On the other hand, lack of awareness on aquarium keeping, lack of technology in breeding for economically important fishes, mass scale production result in serious economic loss. Further, in large scale culture facilities, where aquatic animals are exposed to stressful conditions, poor water quality management, lack of continuous pond aeration, stocking many varieties of fish under one facility, deterioration of environmental conditions and problems related to diseases are some of the major constrains (Prathvi et al., 2013).

1.5. **Disease in aquaculture**

Diseases are usually caused by biological pathogens such as virus, parasite, fungus and bacterium. For the past 10 years, disease is the primary constraint to
many of aquatic species resulting in great economic loss (Qi et al., 2009; Kim et al., 2010). Infectious diseases are always a hazard and accounts for 60 % of the production loss. Sudden changes in the environmental factors (low dissolved oxygen content, fluctuation in water temperature, pH, high concentration of toxic substances such as ammonia, nitrite, hydrogen sulphide and carbon dioxide), high stocking density and poor management lead to spreading of disease (Jeremic et al., 2003). Besides, quite a few disease problems in the ornamental fish are due to the external damages while transporting the fishes (Pandey, 2013). Potential pathogens are able to maintain themselves in the external environment of the animal (water) and proliferate independently in the host animal (Hansen and Olafsen, 1999; Verschuere et al., 2000).

1.6. Diseases in aquaculture - Bacterial pathogens

Next to the viral infection, bacterial infections are one of the most important causes of disease problems in Indian aquaculture (Sahoo et al., 2011). Gram negative bacteria such as Aeromonas, Acinetobacter, Bacteroides, Citrobacter, Flavobacterium, Pseudomonas, Yersinia, Edwardsiella, Aphanomyces and Vibrio are the most common and major bacterial pathogens of fish (Hovda et al., 2007; Kim et al., 2007; Navarrete et al., 2010). Also, Gram positive Streptococcus and, rarely, Carnobacterium have been reported (Baya et al., 1991) as fish pathogens.

Vibrio anguillarum, aetiological agent of classical vibriosis, possesses a wide distribution causing a typical haemorrhagic septicaemia in a wide variety of economically important warm and cold water fishes (Toranzo and Barja, 1990, 1993; Actis et al., 1999). Fenestella asiatica has been implicated as the causative agent of mortality in Tilapia (Oreochromis sp.) (Soto et al., 2010). In Rainbow trout farming, one of the most prominent bacterial diseases is caused by Yersinia ruckeri (Bergh et al., 2008). Plesiomonas shigelloides has been isolated from infected Asian arowana
and Red hybrid tilapia (Jin et al., 2011; Nadirah et al., 2012). *Edwardsiella tarda* is reported as a causative agent of edwardsiellosis that leads to extensive economic loss in a number of commercially important fishes including Puffer fish (Nuccia et al., 2002), Flounder, Eel, Largemouth bass, Japanese flounder (Miwa and mano, 2000), Tilapia sp. (Pirarat et al., 2007) and Turbot (Qin et al., 2007). *Citrobacter freundii* has been isolated from diseased (Citrobacter gastroenteritis) Atlantic salmon, Carp, Rainbow trout and Catfish (Jeremic et al., 2003; Lu et al., 2011).

In addition, *Aeromonas hydrophila* is one of the most common pathogens that affect a wide variety of freshwater fish species and occasionally marine fish (Azza et al., 2009). This bacterium causes haemorrhagic septicaemia, ulceration, abscesses, exophthalmia, abdominal distension, small superficial lesions, local haemorrhages, particularly in the gills and opercula (Kaleeswaran et al., 2011). Indian major carps are the major cultivable fish species in the Indian sub-continent, have been reported to be highly susceptible to *A. hydrophila* (Roberts et al., 1989; Shome et al., 2005). The diseases caused by this pathogen produce considerable economic loss during intensive aquaculture.

1.6.1. **Ornamental fish diseases**

Ornamental fishes are delicate, sensitive and susceptible to various diseases. Poor water quality, temperature fluctuation and poor feed quality are some of the major reasons for the occurrence of diseases in ornamental fish farming. Loss of appetite, deterioration of colour, abnormal swimming behaviour and sluggishness are the general disease symptoms of the diseased fishes. The most commonly observed diseases in both freshwater and marine ornamental fish are columnaris, fin rot, tail rot and white spot disease (Dhayanithi et al., 2012; Pandey, 2013). Carp
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erthrodermatitis, Aeromonas septicaemia, Edwardsielosis, Pseudomonas septicaemia, Flexibacteriosis, bacterial gill disease, Streptococcal septicaemia, infectious abdominal dropsy, furunculosis, Vibrosis, columnaris disease, peduncle disease (fin rot), pasteurellosis, myxobacterial gill and fin infections, epitheliocystis and Mycobacteriosis are some of the reported bacterial diseases in freshwater ornamental fishes (Jeney and Jeney, 1995). Bacterial septicaemia associated anaemia, emaciation, haemorrhagic lesions on the body, dydrops and subsequent mortality due to these diseases are reported in India (Dilip and Dey, 1986), resulting in low price.

According to the most recent review, around 37 bacteria have been reported to be pathogenic for fish (Fryer and Rohovec, 1993). Of these, eight bacterial species have been associated with Carp disease (Jeney and Jeney, 1995). Further, *Citrobacter* infection has been reported in Carp (Karunasagar and Pari, 1992). In general stress and poor water quality are the major reasons for diseases in many of the ornamental fishes including Cichlid variety (Katherine, 2014). Gram negative *Aeromonas salmonicida*, *Yersinia ruckeri* and *Vibrio anguillarum* might cause major diseases in the Cichlid fish (Austin and Austin, 1999). In addition, Sakata (1990) reported that *Aeromonas*, *Plesiomonas* and Enterobacteriaceae are dominant pathogenic species in the freshwater aquaculture.

1.6.2. *Citrobacter* and *Plesiomonas* infections in freshwater fish

Sato *et al.* (1982) reported that *C. freundii* causes diseases in the freshwater fishes. This species comes under Enterobacteriaceae family. The *Citrobacter*, particularly *C. freundii*, have been isolated from Atlantic salmon, Carp, Rainbow trout and Catfish (Baya *et al.*, 1990; Austin *et al.*, 1992; Toranzo *et al.*, 1994; Jeremic *et al.*, 2003; Lu *et al.*, 2011). In India, Karunasagar *et al.* (1992) isolated *C. freundii*
from Carp. The percentage of mortality has been found high in Carp fry (*Cyprinus carpio*) due to *C. freundii* infection. Further it has also been reported in Crucian carp (*Carassius carassius*), fry of Amur (*Ctenopharyngodon idella*) and Roach (*Rutilus rutilus*) (Jeremic et al., 2003).

The symptoms of *C. freundii* infections are typical hemorrhagic septicemia on the skin and internal organs of the fishes. Further infected fish shows anaemic and swelling of the gill filaments, peritonitis and bloody transudate in the abdominal cavity. In addition, the internal organ of fish such as liver colour changed into light-pinkish with petechial haemorrhages, enlarged spleen with rounded edges, greyish colour kidney and inflammation of the intestine have been reported (Jeremic et al., 2003). Besides it has been reported that the *C. freundii* can cause petechial hemorrhages on the gills and developed diffuse bleeding on the skin of Zebra fish (Aijun et al., 2011).

On the other hand, *Plesiomonas shigelloides* is also a potential fish pathogen (Gonzalez et al., 1999). This bacterium is closer to the genus *Proteus* within the family Enterobacteriaceae (Holt et al., 1994). Also they are closely related to *Vibrio* spp. *P. shigelloides* even found in freshwater and estuarine habitat in temperate climate areas (Sanyal et al., 1987; Krovacek et al., 2000). *P. shigelloides* has been reported in Rainbow trout (Cruz et al., 1986). Further Jin et al. (2011) isolated *P. shigelloides* from the kidney samples of Asian arowana and this species has been isolated from 1-2 year old Red hybrid tilapia and caused 40 % mortality (Nadirah et al., 2012). They normally reside in the gastrointestinal tract of warm water fishes, where they could constitute a reservoir of infection (Vandepitte et al., 1980). This
bacterium has also been called “Asian” bacterium because it has been reported highly in Asian countries (Gonzalez, 2003).

1.7. Treatment measures and drawbacks

In recent decades there is a substantial increase in the use of drugs to prevent or control the disease problems in animal production industry. In the last 60 years, globally tonnes of antibiotics have been distributed in the biosphere (Balcazar et al., 2006). Antimicrobial drugs have been used as either prophylactic (preventative), or for growth enhancement (Van den Bogaard and Stobberingh, 2000). At present, in ornamental fish farms the bacterial infections are partially controlled by the application of disinfectants (e.g. hydrogen peroxide and malachite green), anthelmintic agents (e.g. pyrethroid insecticides and avermectins) and antibiotics such as sulfonamides, oxytetracycline, gentamicine, tetracycline, amoxicillin and cefazolin, and/or chemicals such as formalin, sodium chloride, and genoclean (Rawn et al., 2009; Harikrishnan et al., 2010).

There are certain rules for antibiotic application, which includes permissible routes of delivery, dosage, withdrawal times, tolerant limits and species specification. The most common route for the delivery of antibiotics to fish is through feed (coating the antibiotics in regular feed). It has been estimated that 75 % of the antibiotic fed to fish are excreted into the water (Burridge et al., 2010). However, the utility of antimicrobial agents as a control and preventive measure has been questioned due to antimicrobial resistance among pathogenic bacteria (Smith et al., 1994) and antimicrobial accumulation in tissue and environment. However, tetracycline resistance determinants have been detected in commensal bacteria such as Citrobacters isolated from Catfish (Nawaz et al., 2008). Further, it has been reported
that *P. shigelloides* is resistant to amoxicillin/clavulanic acid, ampicillin, chloramphenicol, tetracycline and trimethoprim/sulfamethoxazole. The desire to prevent the establishment of pathogenic bacteria have led the over usage of antimicrobial drugs (Schwarz *et al.*, 2001). Moreover, chemotherapy may kill or inhibit the normal and beneficial microflora of the animal digestive tract and in the culture environment (Sugita *et al.*, 1991; Aly *et al.*, 2008). Thus, to keep a healthy and eco friendly environment it is necessary to think about alternative prophylactics measures.

**1.8. Alternative management**

The increase in bacterial resistance due to antibiotic, their residues in the environment and deprived immune response are becoming global concerns in recent days. To overcome this issue there is a need to develop alternative therapies against bacterial pathogens in animal production, especially in aquaculture. Vaccination is an ideal method for preventing infectious diseases, however it is not a treatment for existing infections and application to the huge population is a big question mark. Moreover, it is very difficult to do the vaccination during mid of the culture period in fish farms. Furthermore, phage therapy has gained much attention for its advantages in preventing and controlling pathogen (Boriea *et al.*, 2014). In recent days, applications of different medicinal plants and herbs or combinations of these compounds have been used to overcome the disease problems. Applications of these plant based compounds have many advantages such as anti-bacterial, anti-fungal, enhance the immune system, hormonal balancing, etc., (Harikrishnan *et al.*, 2011). On the other hand, essential oils are generally recognised as safe substances (GRAS) extracted from plants. Due to their antimicrobial properties, these oils may constitute
alternative prophylactic and therapeutic agents in aquaculture (Gutierrez et al., 2008, 2009). Probiotics have been proved as a suitable prophylactic microbial organism and widely accepted to be used in aquaculture (Gatesoupe, 1999; Verschuere et al., 2000; Irianto and Austin, 2002a; Balcazar et al., 2006). In general probiotic organism enhances the host immune system, competes with pathogenic organism and eliminates the disease causing organism by secreting antibody-like substance. Because of these properties farmers prefer to use probiotic organism in aquaculture to control or prevent the potential pathogens (Gomez-Gil et al., 2000; Aly et al., 2008; Kim and Austin, 2008).

1.9. Probiotics

Application of probiotics is one of the potential tools to prevent diseases and increase the survival rate in aquaculture (Ringo and Gatespoup, 1998). The term, probiotic, means “for life”, originating from the Greek words “pro” and “bios” (Gismondo et al., 1999). The most widely quoted definition was made by Fuller (1989) as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance”. Probiotics have been an area of much interest and research has been carried out for the past 30 years. The original idea about probiotics was formed by Metchnikoff in the early 1900s (Metchnikoff, 1907).

Today probiotics have a quite common place in health promoting “functional foods” for humans, as well as therapeutic, prophylactic and growth supplements in animal production and human health (Mombelli and Gismono, 2000; Ouwehand et al., 2002; Sullivan and Nord, 2002; Senok et al., 2005). Some possible benefits linked to administer the probiotics have already been suggested as: (i) competitive exclusion of pathogenic bacteria, (ii) source of nutrients and enzymatic contribution to
digestion, (iii) direct uptake of dissolved organic material mediated by the bacteria and others are still being investigated as: (iv) enhancement of the immune response against pathogenic microorganisms, (v) antiviral effects (Balcazar et al., 2006).

Different microorganisms have been used as probiotics such as bacteria, yeast or mold. Probiotic bacteria include species of *Lactobacillus* such as *acidophilus*, *sporogenes*, *plantarum*, *rhamnosus*, *delbrueck*, *reuteri*, *fermentum*, *lactus*, *cellobiosus*, *brevis*, *casei*, *farciminis*, *paracasei*, *gasseri*, *crispatus*; species of *Bifidobacterium* including *bifidum*, *infantis*, *adolescentis*, *longum*, *thermophilum*, *breve*, *lactis*, *animalis*, species of *Streptococcus* including *lactis*, *cremoris*, *salivarius*, *intermedius*, *thermophilis*, *diacetylactis*, species of *Leuconostoc mesenteroides*, species of *Pediococcus*; species of *Propionibacterium*; species of *Bacillus*; species of *Enterococcus*; species of *Enterococcus faecium* (Gupta and Garg, 2009). In addition, probiotic yeast and molds are *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Aspergillus niger*, *Aspergillus oryzae*, *Candida pintolopesi* and *Sacaromyces boulardii* (Nayak and Liong, 2011). However, research is still on to find out new species to overcome many issues.

1.9.1. **Use of probiotics in animal industry and agriculture**

Probiotics existed in our traditional foods such as beverages, salty fishes, yogurt, cheeses, etc., (Amara, 2012). Fermented milk might have been the first real use of food containing probiotics (Hosono, 1992). Recently, scientists are searching for new probiotic bacteria from various sources such as milk, curd, yoghurt, fermented food and gastrointestinal tract of healthy fish, etc. From the earlier studies it was clearly understood that our environment has countless beneficial microorganisms. Based on this, the present study concentrated on the investigation of beneficial
bacteria from various sources including healthy ornamental fishes, milk products and food materials.

The beneficial effects of probiotics in animal production have been related to the improvement of metabolic processes of digestion and nutrient utilization by enzymatic activities, increasing the passage rate of digestion and deconjugating bile salts and acids. In pigs, the probiotic feed improved the growth rate, good food conversion ratio and improved survival (Wilcock, 2011). For many years, yeast and yeast containing products have been used as a source of protein and energy in ruminants’ diets (Sretenovic et al., 2008). It has been demonstrated that direct feeding of probiotic such as *Bacillus* may offer an effective alternative to antibiotic and act as a growth promoter in Turkeys (Higgins et al., 2005; Wolfenden et al., 2011). Bozkurt et al. (2011) reported that application of probiotic in the feed improved the egg production rate, egg weight and egg mass and significantly improves the feed conversion ratio of Layer and Broiler hens.

In soil management, probiotics help to retain, deliver and recycle the existing nutrients, thus it minimizes the fertilizer applications. Probiotics fix nutrients into their cell bodies and produce sticky bio-films. This helps to retain vital elements and water in the soil and rhizosphere. In addition, probiotic bacteria also provide and process nitrogen through nitrogen fixation from the air and by cycling higher ammonium nitrogen into nitrates that are used by plants. Such efficient removal of nitrogenous compounds can also be carried out by nitrifying and denitrifying bacteria such as *Thiobacillus* and *Paracoccus*. Additionally, probiotic bacteria are responsible for solubilising phosphate in the soil (Goncalo, 2014).
1.9.2. Probiotics in aquaculture

Based on the intricate relationship of an aquatic organism with the external environment, Verschuere et al. (2000) suggested the definition “a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment”. The first report about the beneficial bacteria was attributed by Gaixa (1889), stating the existence of bacteria in seawater with an inhibitory effect against Vibrio sp. Subsequently, Rosenfeld and Zobell (1947) described a study of antibiotic producing marine microorganisms. Then, the research has been started to develop biological control agents. In aquaculture, probiotics are used for quite a long time from 1900s. However, only in recent few years probiotics have become an integral part of the culture practices for improving growth and disease management. In addition, application of probiotic leads to high production through enhanced growth and disease prevention (Das et al., 2008). Probiotic supplements are commercially available either as monospecies or multispecies for aquaculture practices (Decamp and Moriarty, 2006). Apart from the nutritional and health benefits (Carnevali et al., 2006), probiotics have been used as water additives, can also play a significant role in decomposition of organic matter, reduction of organic nutrition such as nitrogen and phosphorus level. In addition to the above, probiotic eliminates the toxic substance like ammonia, nitrite and hydrogen sulphide (Boyd and Massaut, 1999).

These beneficial microbes affect the host by modulating and stimulating the immune function, improving the microbial balance in the host gut, providing
nutrition, enzymatic contribution in digestion, improving environmental parameters such as water quality and inhibiting pathogenic microorganisms (Verschuere et al., 2000; Balcazar et al., 2006). Further it confers protection against pathogens by competitive exclusion for adhesion sites (Vine et al., 2004a; Chabrillon et al., 2005), production of organic acids (formic acid, acetic acid, lactic acid), hydrogen peroxide and several other compounds such as antibiotics, bacteriocins, siderophores, lysozyme (El-Dakar et al., 2007). Moreover probiotic modulates physiological and immunological responses of the host organisms.

In fish farming the most commonly studied probiotics organism is Gram positive spore forming *Bacillus* spp. (Wang et al., 2008). This species has been shown to possess adhesion abilities, produce bacteriocins (antimicrobial peptides) and provide immunostimulation (Cherif et al., 2001; Cladera et al., 2004; Duc et al., 2004; Barbosa et al., 2005). Other microorganisms such as lactic acid producing bacteria, *Streptococcus, Vibrio, Pseudomonas* and Bifidobacteria have been used as probiotics for aquatic animals to improve the growth and survival rate (Hong et al., 2005; Kim and Austin, 2006; Vine et al., 2006; Balcazar et al., 2007).

**1.9.2.1. Effect of probiotics on fish growth**

Probiotic bacteria are able to colonize in gastrointestinal tract when administered over a long period of time and offer multiple benefits (Balcazar et al. 2006). Diet of Nile tilapia (*Oreochromis niloticus*) has been amended with a probiotic *Streptococcus* strain which significantly increased the protein and lipid content of fish, further it increased the weight of the fish (Lara et al., 2003). Significant improvement in the growth and the survival rate of ornamental fishes include Swordtail (*Xiphophorus helleri, X. maculatus*) and Guppy (*Poecilia reticulata, P.*
sphenops) have been achieved by probiotic *Bacillus subtilis* and *Streptomyces* feed supplement respectively (Ghosh et al., 2008). In general, probiotics have beneficial effect on digestive processes of aquatic animals as probiotic strains synthesize extracellular enzymes such as proteases, amylases and lipases as well as provide growth factors such as vitamins, fatty acids and aminoacids (Balcazar et al., 2006). Therefore nutrients are metabolized and absorbed more efficiently when the feed is supplemented with probiotics (El-Haroun et al., 2006). This has been reflected in the growth of culture organism.

1.9.2.2. Role of probiotics on water quality management

The safe water quality parameters for fish cultivation is 5.7 - 6.3 mg L\(^{-1}\) for dissolved oxygen concentration, 0.36 - 0.42 mg L\(^{-1}\) for ammonia concentration, and pH between 6.3 and 8.2 (El-Haroun et al., 2006). Accumulation of excess feed could deteriorate the surrounding water quality and this would contribute to the higher bacterial population particularly at the base of ponds as reported by Schubert and Pelz (1993). Poor water quality ultimately increases the stress to the cultivable organism by creating the low oxygen, high ammonia and high nitrate. In addition, high water temperature, rough handling, mechanical injury and over-crowding (Shotts and Gratzek, 1984), resulted in a greater susceptibility to bacterial infection (El-Shafai et al., 2004). Overfeeding resulted in high *P. shigelloides* load in water (Karakassis et al., 2000).

To date very few studies characterized the overall microbial communities or potential pathogens associated with ornamental fishes or their water (Sugita et al., 2005; Raja et al., 2006). Laloo et al. (2007) isolated several strains of *Bacillus* from *Cyprinus carpio* and studied its effect on water quality parameter in ornamental fish.
culture and evaluated its antagonistic potential against *A. hydrophila*. It has been suggested that maintaining high levels of probiotics in production ponds can minimize the accumulation of dissolved and particulate organic carbon. Application of water probiotics indirectly improves the fish health by enhancing the water quality parameters. Probiotics modify the bacterial composition of the water and sediments by inhibiting the pathogens. Further, it indirectly increases the production, controls the disease and increasing the beneficial activities in the aquaculture system (Venkateswara, 2007).

1.9.2.3. Probiotics and immunity

Immunity is an important defence mechanism to protect the animal against infection and to maintain homeostasis. One of the most promising methods of controlling diseases in aquaculture is strengthening the defence mechanisms of fish through prophylactic administration of immunostimulants (Robertsen, 1999). Among the numerous beneficial effects of probiotics, modulation of immune system is one of the most important benefits. Probiotics interact with the immune cells such as mononuclear phagocytic cells (monocytes, macrophages) and polymorphonuclear leucocytes (neutrophils) and natural killer (NK) cells to enhance innate immune responses. Further, probiotics can enhance the number of erythrocytes, granulocytes, macrophages and lymphocytes in different fish (Kumar *et al.*, 2008) and increases the immunoglobulin level (Nayak *et al.*, 2007). Application of probiotics improves the non-specific immune system by means of cellular systems, e.g. increase phagocytosis, lysozyme activities (Irianto and Austin, 2002a), antibody production, increasing the chemiluminescent response and superoxide anion production (Sakai, 1998). Pirarat *et al.*, (2006) reported that supplementing the probiotic feed (*L. rhamnosus*) to Tilapia
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*(Oreochromis niloticus)* stimulates the phagocytic activity. Further, probiotics have been reported to improve the respiratory burst of phagocytic cells, which play a central role in the protection of non-specific cell (Panigrahi *et al.*, 2004; Balcazar *et al.*, 2007). *Bacillus subtilis* and *Lacto bacillus* groups can stimulate the respiratory burst activity in the cultured fish (Nikoskelainen *et al.*, 2003; Zhou *et al.*, 2009).

**1.9.2.4. Beneficial effects of probiotics in fish gut ecology**

The gastrointestinal tract of fish is considered as one of the major infection routes for many pathogens (Groff and LaPatra, 2000; Ringo *et al.*, 2003). Indeed, numerous studies have reported that exposure of the epithelium to the pathogens can result in severe tissue damage, characterised by necrotic enterocytes, deteriorated tight junctions, disorganised and damaged microvilli and damage to the lamina propria (Ringo *et al.*, 2007). Application of appropriate probiotic improves the intestinal microbial flora and balance the gut environment which improves food absorption (Parker 1974), digestive enzymes’ activities (Tovar-Ramirez *et al.*, 2004) and reduced pathogenic problems in gastrointestinal tract (Lloyd *et al.*, 1977). According to Conway (1996), a microorganism is able to colonize the gastrointestinal tract when it can persist there. It is a well known fact that the microbiota in the gastrointestinal tract of aquatic animals can be modified by the ingestion of food materials. Therefore, microbial manipulation constitutes a viable tool to reduce or eliminate the incidence of opportunist pathogens (Balcazar, 2002).

The gut is the organ where probiotics not only establish but also execute their functions including immunostimulatory activity. The immune system of the gut is referred to as gut associated lymphoid tissue (GALT). Unlike mammals, fish lack Peyer's patches, IgA and antigen-transporting M cells in the gut (Buddington *et al.*, 1977).
However, many diffusely organized lymphoid cells, macrophages, granulocytes and mucus IgM found in the intestine of fish constitute the immune function (Bakke et al., 2007; Inami et al., 2009). It is also believed that probiotics and/or their components/products interact with GALT and induce the immune response.

1.9.2.5. Administration of probiotics to the aquatic field

Probiotics can be provided to the host or added to its aquatic environment in several ways: (i) addition via live food (Gomez-Gil et al., 1998); (ii) bathing (Austin et al., 1995; Gram et al., 1999); (iii) addition to culture water (Spanggaard et al., 2001); (iv) addition to artificial diet (Rengpipat et al., 2000). Studies clearly showed that the probiotic containing feed must be given to fish continuously to facilitate the retention of probiotic bacteria in the gut, skin and tank water (Joborn, 1998; Kirjavainen et al., 1998; Nikoskelainen et al., 2003). Probiotic supplemented diet resulted in growth performances and feed utilization in common Carp (Cyprinus carpio) and Indian carp (Yanbo and Zirong, 2006).

1.10. Prebiotics

The scientific concept of human gut microbiota modulation by “prebiotics” was introduced in 1995. Prebiotics are carbohydrates which can be classified into monosaccharides, polysaccharides or oligosaccharides. Further, most of the studies conducted with prebiotics includes fructooligosaccharide (Akrami et al., 2013), galactooligosaccharide (Hoseinifar et al., 2013), insulin, mannanoligosaccharide (Merrifield et al., 2010; Song et al., 2014; Daniels and Hoseinifar, 2014) and xyloooligosaccharides (Hoseinifar et al., 2014). Prebiotics act as a growth factor in specific commensal bacteria, that inhibit the adhesion and invasion of pathogenic
microorganisms in the colon epithelium by competing for the same glycoconjugates found on the surface of epithelial cells, altering the pH of the colon, preference to the barrier function, improvement in the production of mucus, production of short chain fatty acids and induce cytokine production (Delcenserie et al., 2009). Among the beneficial changes, it has been reported that prebiotics can elevate fish resistance to pathogens, improve growth performance, feed utilization, lipid metabolism and stimulate immune response through modulation of intestinal microbiota (Daniels and Hosenifar, 2014).

1.11. Probiotics and prebiotics

Combinations of probiotics and prebiotics having beneficial effects to the host combat diseases, improve growth including the size and weight gain and act as alternative antimicrobial compounds (Irianto and Austin, 2002a). Probiotics live in microbial feed additives that modulate gastrointestinal microbial communities whereas prebiotics stimulate the activity or abundance of beneficial gastrointestinal bacteria (Gibson et al., 2004). Both of these have received widespread attention, showing the improved production, health and disease resistance of aquatic animals (Dimitroglou et al., 2010).

Successful isolation and accurate identification of a suspected pathogen is the preliminary step before suggesting the treatment for any bacterial diseases. Characterizing the microbial communities and pathogenic taxa associated with ornamental fish disease would broadly benefit the aquarium industry, aquaculture and public health officials. This study concentrates on the isolation of bacteria from diseased fish and identification of species by biochemical and molecular tools. The study of infectious disease and its preventive measures by using probiotic application
are of significance to the development of aquaculture. The inhibitory effect of beneficial microorganisms against the specific pathogen in aquaculture (Balcazar et al., 2006) and the species (fish) specificity (Zoetendal et al., 2008) are two of the suggested criteria for the selection of probiotic bacteria. Generally, both in vitro and in vivo evaluation procedures are employed for primary screening and assessment of probiotic properties of bacteria. Before using any organism as a probiotics, it is essential to evaluate its potential, origin of strain, acid tolerance and production of antimicrobial substances (Salminen et al., 1998). Apart from this, criteria that determined by safety considerations, methods of production, processing, method of probiotic administration and the location in the body where the microorganisms are expected to be active are followed in the probiotic selection procedure. Based on this, the present study aimed to isolate and characterize the probiotic bacteria from the natural sources and its anti-infective potential was evaluated against the pathogenic (isolated) bacteria by in vitro and in vivo using Koi carp and Kenyi cichlid fish. The fish survival and growth performance, influence on water quality, immune system, intestinal microbiota and intestinal morphology were investigated for their beneficial effect.